# Genomic insights into ruminant evolution: from past to future prospects

Bao Wang [1,2], Lei Chen[3,*], Wen Wang[1,3,4,*]

[1] *State Key Laboratory of Genetic Resources and Evolution*, *Kunming Institute of Zoology*, *Chinese Academy of Sciences*, *Kunming Yunnan* 650223, *China*

[2] *Kunming College of Life Science*, *University of Chinese Academy of Sciences*, *Kunming Yunnan* 650204, *China*

[3] *Center for Ecological and Environmental Sciences*, *Northwestern Polytechnical University*, *Xi'an Shaanxi* 710072, *China*

[4] *Center for Excellence in Animal Evolution and Genetics*, *Chinese Academy of Sciences*, *Kunming Yunnan* 650223, *China*

## ABSTRACT

Ruminants (Ruminantia) are among the most successful herbivorous mammals, exhibiting wide-ranging morphological and ecological characteristics (such as headgear and multichambered stomach) and including various key livestock species (e. g., cattle, buffalo, yak, sheep, and goat). Understanding their evolution is of great significance not only in scientific research but also in applications potential for human society. The rapid growth of genomic resources provides unprecedented opportunities to dissect the evolutionary histories and molecular mechanisms underlying the distinct characteristics of ruminants. Here we summarize our current understanding of the genetic, morphological, and ecological diversity of ruminants and provide prospects for future studies.

**Keywords:** Ruminantia; Genome evolution; Phylogenomics; Traits; Adaptive evolution

## INTRODUCTION

Ruminantia mammals are among the most successful herbivores, and include six families: i. e., Antilocapridae, Bovidae, Cervidae, Giraffidae, Moschidae, and Tragulidae. Ruminantia comprises at least 200 extant species, with Bovidae the most species-rich, consisting of at least 143 species (Heller et al., 2013), including important livestock such as cattle (*Bos taurus*), yak (*Bos grunniens*), sheep (*Ovis aries*), and goat (*Capra hircus*). Ruminants are distributed across extensive habitats, including different latitudes (from tropical to Arctic regions), different altitudes (from plains to plateaus), and different ecological environments (from deserts to rainforests). Moreover, ruminants exhibit several distinct anatomical features, such as headgear and multichambered stomach. Compared with other herbivores, such as horses, the rumen and omasum in ruminants enable more efficient utilization of plant cellulose (Clauss & Rössner, 2014; Janis, 1976; Russell & Rychlik, 2001). This efficient procurement of food energy may be an important reason why ruminants are so prosperous. Ruminants also possess specialized dentition, speed, and considerable variation in body size. Furthermore, they have played an important role in human civilization due to the domestication of several species, such as cattle, buffalo, yak, sheep, and goat. In modern society, these domestic animals remain important for human diet and utilization. Therefore, understanding the evolution and origin

of ruminants is of great importance for scientific research and for application prospects in human society.

In the past decade, we have witnessed the rapid development of genome sequencing technologies and a resultant wealth of knowledge from animal genomes. To date, the genomes of 73 ruminant species have been reported (Table 1), with increasing availability of resequencing and transcriptome data for representative species, like cattle (Chen et al., 2018; Daetwyler et al., 2014; Medugorac et al., 2017; Soubrier et al., 2016), goat (Alberto et al., 2018; Wang et al., 2016), sheep (Alberto et al., 2018; Hu et al., 2019; Naval-Sanchez et al., 2018), and yak (Qiu et al., 2015; Wang et al., 2019a). Most previous studies have only focused on the evolution of individual species by decoding individual genomes and utilizing large-scale resequencing data; e. g.,

high-altitude adaptation studies on yak and Tibetan antelope (Ge et al., 2013; Qiu et al., 2012; Qiu et al., 2015). Nowadays, large-scale sequencing studies of multiple species across high classification orders have provided extraordinary biological discoveries, e.g., avian phylogenomics (Jarvis et al., 2014). We recently launched the Ruminant Genome Project and conducted a large-scale genomic study of ruminants based on integrated analysis of 51 ruminant genomes (44 newly assembled genomes and seven public genomes) and corresponding transcriptome, resequencing, and functional experiments (Chen et al., 2019; Lin et al., 2019; Wang et al., 2019b). The availability of large-scale genomic resources should provide new insights into our understanding of ruminant diversity, evolution, biogeographic patterns, and adaptation mechanisms.

**Table 1  Currently available genome assemblies for ruminant species**

| Family | Subfamily | Species | Common name | Genome size (Gb) | Scaffold N50 (bp) | Contig N50 (bp) | Assembly level | Reference |
|---|---|---|---|---|---|---|---|---|
| Tragulidae | | *Tragulus javanicus* | Java mouse-deer | 2.59 | 14 082 842 | 80 230 | Scaffold | * |
| | | *Tragulus kanchil* | Lesser mouse-deer | 3.05 | 243 497 | 13 850 | Scaffold | Chen et al., 2019 |
| Antilocapridae | | *Antilocapra americana* | Pronghorn | 2.96 | 18 845 065 | 61 698 | Scaffold | Chen et al., 2019 |
| Giraffidae | | *Giraffa camelopardalis* | Giraffe | 2.47 | 3 153 258 | 25 056 | Scaffold | Chen et al., 2019 |
| | | *Giraffa tippelskirchi* | Masai giraffe | 2.71 | 212 164 | 47 894 | Scaffold | Agaba et al., 2016 |
| | | *Okapia johnstoni* | Okapi | 2.88 | 111 538 | 39 571 | Scaffold | Chen et al., 2019 |
| Cervidae | Cervinae | *Axis porcinus* | Hog deer | 2.68 | 20 764 858 | 172 761 | Scaffold | * |
| | Cervinae | *Cervus albirostris* | White-lipped deer | 2.69 | 3 769 372 | 39 627 | Scaffold | Chen et al., 2019 |
| | Cervinae | *Cervus elaphus* | Red deer | 3.44 | 107 358 006 | 7 944 | Chromosome | Bana et al., 2018 |
| | Cervinae | *Elaphurus davidianus* | Pere David's deer | 2.58 | 2 844 142 | 59 950 | Scaffold | Zhang et al., 2018 |
| | Hydropotinae | *Hydropotes inermis* | Chinese water deer | 2.53 | 13 818 975 | 131 446 | Scaffold | Wang et al., 2019 |
| | Muntiacinae | *Muntiacus crinifrons* | Black muntjac | 2.68 | 1 305 444 | 8 265 | Scaffold | Chen et al., 2019 |
| | Muntiacinae | *Muntiacus muntjak* | Indian muntjac | 2.7 | 1 258 210 | 23 470 | Scaffold | Chen et al., 2019 |
| | Muntiacinae | *Muntiacus reevesi* | Chinese muntjac | 2.6 | 1 221 377 | 72 382 | Scaffold | Chen et al., 2019 |
| | Odocoileinae | *Capreolus capreolus* | Western roe deer | 2.79 | 10 458 | 4 167 | Scaffold | Kropatsch et al., 2013 |
| | Odocoileinae | *Odocoileus hemionus* | Mule deer | 2.34 | 838 758 | 113 295 | Scaffold | Russell et al., 2019 |
| | Odocoileinae | *Odocoileus virginianus* | White-tailed deer | 2.38 | 850 721 | 122 019 | Scaffold | Seabury et al., 2011 |
| | Odocoileinae | *Rangifer tarandus* | Reindeer | 2.9 | 89 062 | 77 671 | Scaffold | Li et al., 2017 |
| Moschidae | | *Moschus berezovskii* | Forest musk deer | 2.81 | 2 509 225 | 57 706 | Scaffold | Chen et al., 2019 |
| | | *Moschus chrysogaster* | Alpine musk deer | 4.97 | 100 428 | 3 769 | Scaffold | Wang et al., 2019 |
| | | *Moschus moschiferus* | Siberian musk deer | 3.07 | 11 728 851 | 34 785 | Scaffold | * |

| Family | Subfamily | Species | Common name | Genome size (Gb) | Scaffold N50 (bp) | Contig N50 (bp) | Assembly level | Reference |
|--------|-----------|---------|-------------|------------------|-------------------|-----------------|---------------|-----------|
| Bovidae | Aepycerotinae | *Aepyceros melampus* | Impala | 2.63 | 344 542 | 82 459 | Scaffold | Chen et al., 2019 |
| | Alcelaphinae | *Alcelaphus buselaphus* | Hartebeest | 3.88 | 12 034 | 889 | Scaffold | Chen et al., 2019 |
| | Alcelaphinae | *Beatragus hunteri* | Hirola | 2.7 | 69 303 | 57 444 | Scaffold | * |
| | Alcelaphinae | *Connochaetes taurinus* | Blue wildebeest | 2.64 | 366 224 | 70 608 | Scaffold | Chen et al., 2019 |
| | Alcelaphinae | *Damaliscus lunatus* | Topi | 3.13 | 1 166 796 | 38 843 | Scaffold | Chen et al., 2019 |
| | Antilopinae | *Procapra przewalskii* | Przewalski＇s gazelle | 2.69 | 5 522 907 | 10 405 | Scaffold | Chen et al., 2019 |
| | Antilopinae | *Antidorcas marsupialis* | Springbok | 3.02 | 694 905 | 42 749 | Scaffold | Chen et al., 2019 |
| | Antilopinae | *Eudorcas thomsonii* | Thomson＇s gazelle | 2.9 | 1 581 717 | 137 476 | Scaffold | Chen et al., 2019 |
| | Antilopinae | *Litocranius walleri* | Gerenuk | 2.98 | 3 126 223 | 62 351 | Scaffold | Chen et al., 2019 |
| | Antilopinae | *Madoqua kirkii* | Kirk＇s dik-dik | 2.65 | 27 730 | 27 722 | Scaffold | Chen et al., 2019 |
| | Antilopinae | *Nanger granti* | Grant's gazelle | 3.03 | 528 456 | 7 454 | Scaffold | Chen et al., 2019 |
| | Antilopinae | *Neotragus moschatus* | Suni | 2.68 | 952 090 | 59 277 | Scaffold | Chen et al., 2019 |
| | Antilopinae | *Neotragus pygmaeus* | Royal antelope | 2.9 | 363 895 | 30 751 | Scaffold | Chen et al., 2019 |
| | Antilopinae | *Oreotragus oreotragus* | Klipspringer | 3.25 | 339 390 | 42 127 | Scaffold | Chen et al., 2019 |
| | Antilopinae | *Ourebia ourebi* | Oribi | 2.52 | 1 259 | 1 259 | Scaffold | Chen et al., 2019 |
| | Antilopinae | *Raphicerus campestris* | Steenbok | 3.14 | 537 161 | 25 050 | Scaffold | Chen et al., 2019 |
| | Antilopinae | *Saiga tatarica* | Saiga antelope | 2.88 | 6 453 | 6 406 | Scaffold | * |
| | Bovinae | *Bison bison* | American bison | 2.83 | 7 192 658 | 19 971 | Scaffold | * |
| | Bovinae | *Bison bonasus* | European bison | 2.58 | 4 690 000 | 14 530 | Scaffold | Wang et al., 2017a |
| | Bovinae | *Bos frontalis* | Gayal | 2.85 | 2 737 757 | 14 405 | Scaffold | Wang et al., 2017b |
| | Bovinae | *Bos grunniens* | Domestic yak | 2.83 | 114 386 978 | 44 716 738 | Chromosome | Qiu et al., 2012 |
| | Bovinae | *Bos indicus* | Zebu cattle | 2.67 | 106 310 653 | 28 375 | Chromosome | Canavez et al., 2012 |
| | Bovinae | *Bos indicus x Bos taurus* | Hybrid cattle | 2.68 | 104 466 507 | 26 764 281 | Chromosome | * |
| | Bovinae | *Bos mutus* | Wild yak | 2.65 | 1 407 960 | 22 822 | Scaffold | * |
| | Bovinae | *Bos taurus* | Cattle | 2.72 | 103 308 737 | 25 896 116 | Chromosome | Bovine Genome et al., 2009 |
| | Bovinae | *Bubalus bubalis* | Water buffalo | 2.66 | 117 219 835 | 22 441 509 | Chromosome | Mintoo et al., 2019 |
| | Bovinae | *Syncerus caffer* | African buffalo | 2.93 | 2 316 376 | 52 316 | Scaffold | Chen et al., 2019 |
| | Bovinae | *Tragelaphus buxtoni* | Mountain nyala | 3.27 | 7 367 | 1 286 | Scaffold | Chen et al., 2019 |
| | Bovinae | *Tragelaphus eurycerus* | Bongo | 2.96 | 12 574 | 1 974 | Scaffold | Chen et al., 2019 |
| | Bovinae | *Tragelaphus imberbis* | Lesser kudu | 4.04 | 1 775 046 | 12 858 | Scaffold | Chen et al., 2019 |
| | Bovinae | *Tragelaphus oryx* | Common eland | 2.84 | 4 043 025 | 1 262 | Scaffold | Chen et al., 2019 |
| | Bovinae | *Tragelaphus scriptus* | Bushbuck | 2.87 | 890 554 | 28 350 | Scaffold | Chen et al., 2019 |
| | Bovinae | *Tragelaphus spekii* | Sitatunga | 3.73 | 46 427 | 9 477 | Scaffold | Chen et al., 2019 |
| | Bovinae | *Tragelaphus strepsiceros* | Greater kudu | 2.88 | 511 483 | 33 649 | Scaffold | Chen et al., 2019 |
| | Caprinae | *Ammotragus lervia* | Barbary sheep | 2.65 | 1 301 762 | 52 017 | Scaffold | Chen et al., 2019 |
| | Caprinae | *Capra aegagrus* | Wild goat | 2.83 | 91 317 560 | 19 347 | Chromosome | Dong et al., 2015 |

| Family | Subfamily | Species | Common name | Genome size (Gb) | Scaffold N50 (bp) | Contig N50 (bp) | Assembly level | Reference |
|---|---|---|---|---|---|---|---|---|
| | Caprinae | *Capra hircus* | Goat | 2.92 | 87 277 232 | 26 244 591 | Chromosome | Bickhart et al., 2017 |
| | Caprinae | *Capra ibex* | Alpine ibex | 2.7 | 61 905 114 | 380 983 | Scaffold | Chen et al., 2019 |
| | Caprinae | *Capra sibirica* | Siberian ibex | 2.73 | 15 190 720 | 376 582 | Scaffold | * |
| | Caprinae | *Hemitragus hylocrius* | Nilgiri tahr | 2.72 | 85 340 | 66 552 | Scaffold | * |
| | Caprinae | *Ovis ammon* | Argali | 2.64 | 72 289 505 | 151 569 | Scaffold | Chen et al., 2019 |
| | Caprinae | *Ovis aries* | Sheep | 2.87 | 107 697 089 | 2 572 683 | Chromosome | Jiang et al., 2014 |
| | Caprinae | *Ovis canadensis* | Bighorn sheep | 2.86 | 69 397 | 55 973 | Scaffold | * |
| | Caprinae | *Pseudois nayaur* | Bharal | 2.58 | 21 385 | 20 979 | Scaffold | Chen et al., 2019 |
| | Cephalophinae | *Cephalophus harveyi* | Harvey's duiker | 2.82 | 365 466 | 61 368 | Scaffold | Chen et al., 2019 |
| Bovidae | Cephalophinae | *Philantomba maxwellii* | Maxwell's duiker | 3.15 | 383 899 | 11 377 | Scaffold | Chen et al., 2019 |
| | Cephalophinae | *Sylvicapra grimmia* | Common duiker | 3.15 | 583 330 | 9 720 | Scaffold | Chen et al., 2019 |
| | Hippotraginae | *Hippotragus niger* | Sable antelope | 2.6 | 4 586 323 | 45 501 | Scaffold | Koepfli et al., 2019 |
| | Hippotraginae | *Oryx gazella* | Gemsbok | 2.76 | 1 579 191 | 19 498 | Scaffold | Chen et al., 2019 |
| | Pantholopinae | *Pantholops hodgsonii* | Tibetan antelope | 2.7 | 2 772 860 | 18 674 | Scaffold | Ge et al., 2013 |
| | Reduncinae | *Kobus ellipsiprymnus* | Defassa waterbuck | 2.9 | 779 552 | 26 169 | Scaffold | Chen et al., 2019 |
| | Reduncinae | *Redunca redunca* | Bohor reedbuck | 2.72 | 423 407 | 39 382 | Scaffold | Chen et al., 2019 |

*: These genomes can be accessed from the NCBI website (https://www.ncbi.nlm.nih.gov/assembly/).

In this review, we first summarize current knowledge of ruminant evolution learned from the genomic era, especially phylogenetic relationships, genetic basis and evolution of complex traits, genetic basis underlying local adaptations, and domestication of livestock ruminants. We then provide an outlook for future research on ruminants given the rapid advances in the accumulation of genomic resources and increasing efforts in functional genomics.

## PHYLOGENETIC RELATIONSHIPS OF RUMINANTS

Phylogeny is essential to biology not only because it reflects the evolutionary history of species or lineages and divergent events, but also provides a framework for tracing the evolution of distinct complex characteristics. In previous studies, phylogenetic trees of ruminants were constructed based on morphological data (e. g., teeth and skeletons), fragments of nuclear DNA, or complete mitochondria DNA (Bibi, 2013; Decker et al., 2009; Gatesy et al., 1992; Hassanin & Douzery, 2003; Hassanin et al., 2012). However, controversies in these phylogenetic trees remain, resulting in different placements at the genus, subfamily, and even family level. To resolve these issues, Chen et al. (2019) provided a robust phylogenetic tree based on whole genome data from 51 ruminants, clarifying

most of the controversies in ruminant phylogeny. Here, using all available genomes (Table 1), we reconstructed a simplified phylogenetic tree (Figure 1). In previous studies, Antilocapridae was considered an outgroup to all other pecorans (all ruminants, excluding Tragulidae) based on mtDNA (Bibi, 2013; Hassanin et al., 2012; Hernández Fernández & Vrba, 2005). Moschidae was placed at the base of Pecora (Janis, 1987; Janis & Theodor, 2014; Webb & Taylor, 1980) or as a sister group to Bovidae based on morphological data (Sánchez et al., 2010), or proposed as a sister group of Cervidae or Bovidae based on mtDNA (Bibi, 2014; Dos Reis et al., 2012; Hassanin & Douzery, 2003; Hassanin et al., 2012). The whole genome tree confirms the sister-group relationship between Antilocapridae and Giraffidae, and Moschidae as a sister group with Bovidae. In addition, some controversies in Bovidae (Hernández Fernández & Vrba, 2005) are also resolved, with Reduncinae confirmed as a sister group of Caprinae, Alcelaphinae, and Hippotraginae.

The above phylogenic controversies may be due to incomplete linage sorting (ILS) or to introgression between linages. A high level of phylogenetic heterogeneity is observed at the family level of ruminants, showing that only 21.5% of sample genomic windows are consistent with the topology of
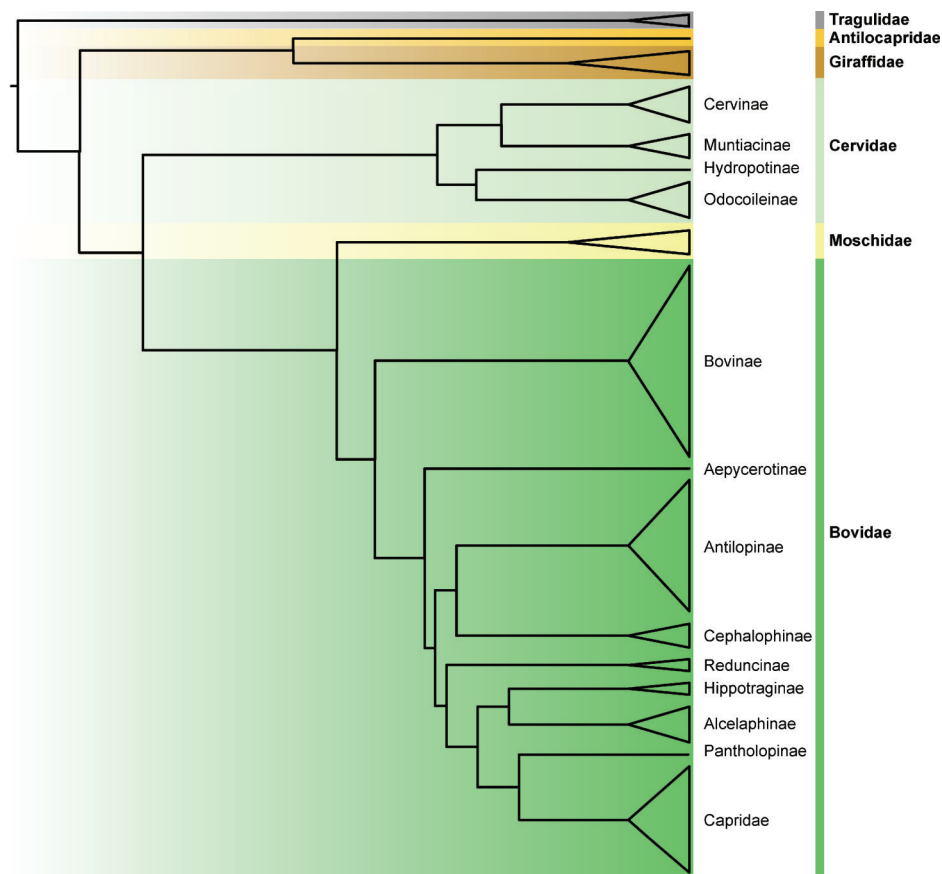
**Figure 1 Phylogenetic relationship among ruminants**

Phylogenic tree of ruminants is presented with species within same families and subfamilies collapsed. Species used in this tree are listed in Table 1, and the phylogenetic relationship is mainly based on Chen et al. (2019).

the whole genome tree (Chen et al., 2019). These phylogenetic disagreements can primarily be explained by ILS, resulting from the rapid radiation of ruminant families during the Miocene (Chen et al., 2019). In addition, a strong gene flow signal has been reported among Giraffidae, Cervidae, Bovidae, and Moschidae (Chen et al., 2019), implying ancestral introgressions may also partially account for the phylogenic debates. In particular, pervasive introgression among *Bos* species has been reported from other studies (Wang et al., 2018a; Wu et al., 2018), which provides support for introgression effects on the phylogenetic relationships of ruminants.

## EVOLUTION OF IMPORTANT TRAITS

Most traits are consequences of long-term evolution and form the basis of biodiversity. As one of the most successful mammalian linages, ruminants have evolved extensive and unique morphological traits, such as headgear, multichambered stomach, substantial variation in body size, cold (Arctic) adaptation (reindeer), and high-altitude adaptation. The availability of large-scale genomic data,

together with transcriptomics and functional genomics, has enabled investigations into the genetic basis and evolution of complex traits in ruminants.

### Evolution of headgear

Ruminants are the only mammalian group with osseous headgear (Davis et al., 2011). Headgear is not only used in self-defense against predators but also in intraspecies competition for mates and territories (Bubenik & Bubenik, 2012; Davis et al., 2011). Four families of ruminants have headgear, including Giraffidae, Antilocapridae, Cervidae, and Bovidae. Headgear in different families exhibits distinct morphological characteristics, such as ossicones in giraffids, pronged horns in pronghorns, antlers in cervids, and horns in bovids (Bubenik & Bubenik, 2012). The genetic mechanisms involved in headgear have long been a topic of interest to evolutionary biologists (Aristotle, 1991; Goss, 1983).

The expression profiles, phylogenies, and convergent headgear losses support a single evolutionary origin of ruminant headgear (Chen et al., 2019; Wang et al., 2019b). The highly or specially expressed genes in horns and antlers are mostly co-expressed in bone, skin, nerve tissue, and testis, and many positively selected genes are associated with

conserved elements involved in neural functions (Wang et al., 2019b). The headgear in ruminants likely originated from neural crest stem cells, which diverged into horns, antlers, ossicones, and pronged horns in the bovids, cervids, giraffids, and pronghorns, respectively. The loss of headgear in Moschidae and Hydropotinae was likely due to convergent pseudogenization of the gene *RXFP2* (Wang et al., 2019b).

Interestingly, antlers in the Cervidae have evolved further remarkable features. Professor Richard Goss from the University of Brown wrote, "*The antlers of deer are so improbable that if they had not evolved in the first place they would never have been conceived even in the wildest fantasies of the most imaginative biologists*" (Goss, 1983). Deer antlers are the only completely regenerative organ found in mammals. They exhibit extremely rapid growth rates (~1.7 cm/day in red deer) that can even surpass cancerous tissue growth in cell proliferation (Goss, 1983), although deer experience low rates of cancer (Griner, 1983; Lombard & Witte, 1959). Wang et al. (2019b) showed that antler-specific expressed genes are enriched in the axon guidance pathway, suggesting that antler growth may be involved in neural processes. Strikingly, their results also revealed that proto-oncogenes (*FOS*, *REL*, *FAM83A*) and tumor suppression genes are positively selected in cervids, thus explaining the high growth rate of deer antlers but low cancer rate in cervids. Understanding the genetic basis of rapid antler regeneration could provide new insights into the regeneration of mammalian organs and oncogenesis.

### Evolution of multichambered stomach

The ruminant digestive system plays important ecological and functional roles in evolution. The appearance of the rumen and its close interaction with microorganisms allows ruminants to gain energy efficiently, thus providing unique evolutionary advantages and promoting the prosperity and diversity of ruminant groups. Compared with non-ruminants (e.g., horses), ruminants can achieve higher utilization of plant fiber due to their unique forestomach fermentation system (Janis, 1976). Most previous studies have focused on the interaction between microorganisms and the rumen (Bovine Genome Sequencing and Analysis Consortium et al., 2009; Seshadri et al., 2018; Stewart et al., 2018; Zhang et al., 2016). However, little is known about the origin and evolution of the multichambered stomach.

To investigate the origin and evolution of the ruminant stomach, Chen et al. (2019) compared gene expression profiles from 516 samples covering 50 tissues of sheep, together with large-scale genomic data. The expression profiles suggested that the rumen, reticulum, and omasum may have originated from the esophagus, as also suggested by Warner (1958) and Xiang et al. (2016), whereas the abomasum was closer to the intestine. Newly evolved genes also seem to have played an important role in the evolution of the rumen. Seven newly evolved genes identified at the ancestor of ruminants are specially expressed in the rumen, two of which, *PRD-SPRRII* and *TCHHL2*, are structural genes (Chen et al., 2019; Jiang et al., 2014). *PRD-SPRRII* is related

to the cornification of the keratin-rich surface of the rumen, and *TCHHL2* plays a role in cross-linking keratins at the rumen surface (Jiang et al., 2014). The omasum is a newly evolved organ in pecorans, resembling the rumen in structure and function (Millen et al., 2016). Among the 75 genes specifically and highly expressed in the omasum compared with other organs, one gene is newly evolved (*LOC101107119*) and another (*SCNN1D*) exhibits pecoran-specific amino acid changes (Chen et al., 2019). In addition, four genes (i.e., *SIM2*, *PAX9*, *KCNK5*, and *DENND2C*) show pecoran-specific conserved non-exonic elements (CNEs) within their immediate upstream/downstream 10 kb regions (Chen et al., 2019). Based on gene family analysis, the lysozyme *c* family is also expanded in ruminants, containing ten or more copies (Chen et al., 2019; Irwin, 2015), whereas other outgroup mammals have only one or a few copies. Furthermore, most expanded copies of lysozyme *c* are expressed in the abomasum, suggesting adaptation to its microbe-rich environment.

### Evolution of body size

Ruminants exhibit extensive variation in body size, from 2 kg (e.g., lesser mouse deer, *Tragulus kanchil*) to 1 200 kg (e.g., giraffe, *Giraffa camelopardalis* and African buffalo, *Syncerus caffer*) (Castelló, 2016). At the family level, body size is largest in Giraffidae, followed by medium-sized Antilocapridae and Cervidae, and relatively small-sized Tragulidae and Moschidae (Clauss et al., 2003). Bovidae exhibits varied body size, with Bovinae generally being the largest (Castelló, 2016). Ruminants living in open grasslands also tend to be larger, which can help in resisting predators and adapting to changes in the environment. Ruminants living in the jungle tend to be smaller in size, which allows for more flexible movement and thus predator avoidance.

Genes associated with body size have significantly higher non-synonymous substitution rates in large- and small-sized bovid species (Chen et al., 2019). Specifically, six genes (i.e., *CXCL13*, *RNF115*, *NPNT*, *KL*, *SLC9A3R1*, and *MSTN*) are associated with increased body size and five genes (i.e., *SBDS*, *BMP3*, *LRRN3*, *NFATC3*, and *SMARCAL1*) are related to reduced body size via regulation of bone and muscle development. Several genes also have functional support from other studies. For example, *SLC9A3R1* plays a role in osteogenesis by mineralizing osteoblasts, with disruption of this gene resulting in reduced body weight in mice (Shenolikar et al., 2002). *MSTN* regulates muscle cell growth and differentiation (Sartori et al., 2013), and mutations in *MSTN* can affect muscle mass in livestock species such goat, sheep, and cattle (Grobet et al., 1997; Li et al., 2016; Luo et al., 2014; Wang et al., 2018b) and other mammals (Bi et al., 2016; Gu et al., 2016). *SBDS* serves an important role in cell proliferation and is a causal gene of Shwachman-Diamond syndrome in humans (Boocock et al., 2003), which is characterized by short stature and skeletal abnormalities. For individual species, the special stature of giraffes enables access to food and is an adaptation to tropical grassland. Those genes associated with bone development with giraffe-specific

mutations are involved in the transforming growth factor-b (TGF-b), Hedgehog, Notch, Wnt, and FGF signaling pathways (Agaba et al., 2016; Chen et al., 2019).

## Reindeer adaptation to Arctic regions

Reindeer (*Rangifer tarandus*) are naturally distributed across Arctic and sub-Arctic regions, and consequently face numerous challenges, including severe cold, limited food availability, and prolonged periods of light or darkness (Blix, 2016). To adapt to these environments, reindeer have evolved several strategies, including special lipid metabolism to avoid heat loss and to induce circadian arrhythmicity for long periods of light or dark (Lu et al., 2010; Stokkan et al., 2007; Van Oort et al., 2005). Based on detailed comparative genomic analysis, Lin et al. (2019) identified two positively selected genes (i. e., *CYP27B1* and *POR*) involved in the vitamin D metabolism pathway. Functional experiments have also shown that enzymes encoded by these two genes exhibit much higher catalytic activity than that of their orthologs in goat and roe deer (Lin et al., 2019). Eight genes with reindeer-specific mutations have been reported to be involved in the circadian rhythm pathway, with four being rapidly evolving genes. Among these genes, Pro1172Thr (P1172T) mutation in *PER2* results in loss of binding ability with CRY1, which can cause arrhythmicity (Lin et al., 2019).

## Adaptation to high altitude

Ruminants are distributed across a wide range of habitats, including high-altitude regions such as the Tibetan Plateau, with risks of hypobaric hypoxia. These ruminants include the yak, Tibetan sheep, and Tibetan goat, which are three key livestock species serving as sources of meat and fiber for Tibetan inhabitants (Hu et al., 2019; Qiu et al., 2012; Wang et al., 2016). In addition, the Tibetan antelope (*Pantholops hodgsonii*) and Marco Polo sheep (*Ovis ammon polii*) also exhibit adaptation to high altitude (Ge et al., 2013; Yang et al., 2017). Therefore, understanding the genetic basis and mechanisms underlying high-altitude adaptation is of great value in many scientific fields.

Genetic variation related to transcription factors, oxygen sensors, and target genes has been exploited to detect signals of selection in ruminant species such as the Tibetan sheep (Hu et al., 2019), Tibetan goat (Wang et al., 2016), yak (Qiu et al., 2012), and Tibetan antelope (Ge et al., 2013). In yak, three genes, identified as positively selected in response to hypoxia, are involved in hypoxia-inducible factor-1α (Hif-1α), including two important regulators (Adam17 and Arg2) and one target gene (*Mmp3*) (Qiu et al., 2012). In Marco Polo sheep, several genes associated with hypoxia response have been identified, including ryanodine receptor 1 (*RYR1*) and purinergic receptor P2X and ligand-gated ion channel 3 (*P2RX3*) (Yang et al., 2017). In addition to whole genome analysis in yak, Marco Polo sheep, and Tibetan antelope, resequencing analysis between low- and high-altitude ruminant populations has also identified candidate genes associated with hypoxia response, especially the HIF-1 pathway (Hu et al., 2019; Wang et al., 2016), including one

gene (*NOXA1*) in goats (Wang et al., 2016) and eight genes (*EPO*, *TLR4*, *PIK3CA*, *PRKCA*, *EGLN3*, *EGLN2*, *IFNGR2*, and *CUL2*) with strong signals in sheep (Hu et al., 2019). Although candidate genes under positive selection can contribute to high-altitude adaptation, introgression may also play a crucial role. For instance, genes involved in the response-to-hypoxia pathway (e. g., *EGLN1*, *EGLN2* and *HIF3a*) are introgressed from yak to Tibetan cattle, which may be responsible for their high-altitude adaptation (Wu et al., 2018). Of these genes, *EGLN1* is reported to be associated with high-altitude adaptation in Tibetans (Simonson et al., 2010). Therefore, these positively selected genes may provide valuable insights into plateau medicine.

## DOMESTICATION EFFECTS ON RUMINANT LIVESTOCK

The importance of ruminants is also highlighted by several major livestock species, including goat, sheep, cattle, and yak. Domestication of livestock ruminants was a key factor in the transition from hunter-gather societies to agricultural civilizations. Under intense artificial selection, domesticated livestock species have experienced extensive morphological or physiological changes compared to their wild relatives, including shadow color coat, floppy ears, shorter muzzles, smaller brain and cranial capacity, and tameness, which are referred to as domestication syndromes (Wilkins et al., 2014).

It has been hypothesized that domestication syndromes predominantly resulted from mild neural crest cell (NCC) deficits during embryonic development (Wilkins et al., 2014). Several recent studies have revealed that selected candidate regions under domestication of cattle, yak, sheep, and goat are enriched in pathways related to brain development or neurobehavioral functioning, such as NCCs (Alberto et al., 2018; Naval-Sanchez et al., 2018; Qanbari et al., 2014; Qiu et al., 2015). For example, neural genes (e. g., *TMEM132D*, *CACNA1C*, *NRXN1*, and *NPAS3*) show signals of positive selection in cattle (Qanbari et al., 2014). Thirty genes in yak show signals of selection involved in brain and neuronal development, 19 of which are associated with behavior (Qiu et al., 2015). In addition, five genes are associated with the nerve system in sheep and goat, which showed convergent evolution (Alberto et al., 2018). These genes associated with the brain and nerve system under pressure from artificial selection may explain tameness or aggressiveness in livestock species.

In addition to major livestock species, reindeer were also domesticated by sub-Arctic people. Although cervid species are usually cautious and sensitive, reindeer are very tame and are the only domesticated species in the cervid family. Lin et al. (2019) identified reindeer-specific mutations in genes associated with NCCs to explain their tameness, and observed that genes involved in development (*MSX2*, *ID3*, *BCAT1*, *CAD6*, and *CAD11*), migration (*TCOF1*, *BCAT1*, *NOTCH2*, and *NOTCH3*), and differentiation (*COL2A1*, *KIT*, and *SI*) of NCCs were either rapidly evolving or demonstrated reindeer-specific mutations.

## PERSPECTIVES

Traditional biological methods are often unable to solve the genetic basis of the evolution of complex traits across higher taxa at the family, order, or even class level. To address this issue, we propose a new approach called evolutionary genotype-phenotype systems biology (eGPS), which compares the evolution of many species using large-scale genome, transcriptome, regulatome, metabolome, and proteome data and systematically integrates evidence from genetic factors, developmental network evolution, and phenotypes to analyze the genetic basis of complex animal traits, especially those with evolutionary significance. Using this approach, we could resolve the genetic basis of complex traits and determine key regulatory genes or pathways. Functional experiments could verify these genetic factors, and thus facilitate further investigation on the evolutionary mechanisms of complex traits and their potential application. The Ruminant Genome Project has served as a good example of eGPS study with large-scale multi-omics data.

The rapid development of sequencing technology, especially long-read and Hi-C technologies, has enabled researchers to obtain high-quality reference genomes with long continuous contigs and scaffold size, even to the chromosome level. High-quality assemblies should promote additional studies on ruminants, including chromosome evolution, accurate identification of new functional elements (e. g., regulatory elements and new genes), and functional validation using gene editing technology such as *CRISPR/Cas9*. Chromosome numbers in *Muntiacus* species demonstrate considerable variations due to chromosome fusion; for example, *Muntiacus reevesi* has a karyotype of 2$n$= 46, *Muntiacus vaginalis* has a karyotype of 2$n$=6 and 7, and *Muntiacus crinifrons* has a karyotype of 2$n$=8 and 9 in females and males, respectively (Shi, 1983; Wurster & Benirschke, 1967, 1970). High-quality reference genomes will help to reveal the genetic mechanisms underlying the recurrent fusion of chromosomes in muntjacs and its effects on the interaction between genes flanking the fusion regions, and thus facilitate understanding of speciation driven by chromosome variation. New regulatory elements and genes may be crucial in adaptive phenotypic diversification. Availability of high-quality genome data should facilitate identification of these new functional elements, thus revealing the potential genetic basis underlying adaptations. For casual or functional mutations identified using large-scale data among linages, *CRISPR/Cas9* experiments could be utilized for verification using model organisms or key livestock species, such as sheep and goats.

Additionally, genomic resources and comparative analyses of ruminants are likely to contribute to animal husbandry (Figure 2). Comparative analysis between domestic livestock species and wild species has identified a series of candidate genes associated with domestication or economically valuable traits, such as meat quality and milk production (Alberto et al., 2018). These artificially selected genes can provide new targets for animal breeding and improvement. In the future,

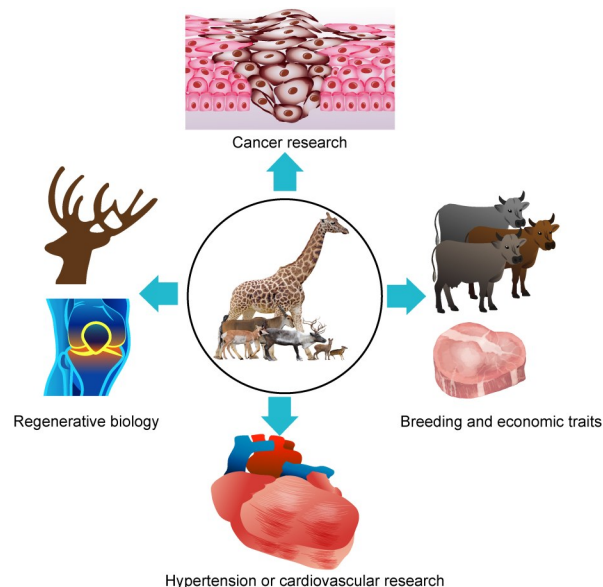gene editing technology (such as *CRISPR/Cas9*) may be applicable to quickly improve traits of livestock.



**Figure 2  Possible applications of future ruminant research**
Studies on ruminants could be applied in cancer research, regenerative biology, hypertension, and cardiovascular research, as well as breeding of livestock.

In addition to application in agriculture, ruminants could serve as animal models for medical research and thus provide valuable insights into medical treatments and even clinical studies (Figure 2). Further research on deer antlers may offer unprecedented clues on regenerative biology and oncogenesis. Moreover, study on giraffes may provide insight into hypertension or cardiovascular research. Overall, increasing our understanding of the genetic basis and mechanisms underlying certain ruminant traits could benefit human society in many ways.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

W. W. and L. C. proposed the ideas and revised the manuscript. B. W. collected data, reviewed the literature, and drafted the manuscript. All authors read and approved the final version of the manuscript.

## REFERENCES

Agaba M, Ishengoma E, Miller WC, Mcgrath BC, Hudson CN, Bedoya

Reina OC, Ratan A, Burhans R, Chikhi R, Medvedev P, Praul CA, Wu-Cavener L, Wood B, Robertson H, Penfold L, Cavener DR. 2016. Giraffe genome sequence reveals clues to its unique morphology and physiology. *Nature Communications*, **7**: 11519.

Alberto FJ, Boyer F, Orozco-Terwengel P, Streeter I, Servin B, De Villemereuil P, Benjelloun B, Librado P, Biscarini F, Colli L, Barbato M, Zamani W, Alberti A, Engelen S, Stella A, Joost S, Ajmone-Marsan P, Negrini R, Orlando L, Rezaei HR, Naderi S, Clarke L, Flicek P, Wincker P, Coissac E, Kijas J, Tosser-Klopp G, Chikhi A, Bruford MW, Taberlet P, Pompanon F. 2018. Convergent genomic signatures of domestication in sheep and goats. *Nature Communications*, **9**(1): 813.

Aristotle A. 1991. History of Animals. Cambridge, MA: Harvard University Press.

Bana NA, Nyiri A, Nagy J, Frank K, Nagy T, Steger V, Schiller M, Lakatos P, Sugar L, Horn P, Barta E, Orosz L. 2018. The red deer *Cervus elaphus* genome CerEla1.0: sequencing, annotating, genes, and chromosomes. *Molecular Genetics and Genomics*, **293**(3): 665–684.

Bi Y, Hua Z, Liu X, Hua W, Ren H, Xiao H, Zhang L, Li L, Wang Z, Laible G, Wang Y, Dong F, Zheng X. 2016. Isozygous and selectable marker-free MSTN knockout cloned pigs generated by the combined use of CRISPR/Cas9 and Cre/LoxP. *Scientific Reports*, **6**: 31729.

Bibi F. 2013. A multi-calibrated mitochondrial phylogeny of extant bovidae (Artiodactyla, Ruminantia) and the importance of the fossil record to systematics. *BMC Evolutionary Biology*, **13**: 166.

Bibi F. 2014. Assembling the ruminant tree: combining morphology, molecules, extant taxa, and fossils. *Zitteliana*, **32**: 197–211.

Bickhart DM, Rosen BD, Koren S, Sayre BL, Hastie AR, Chan S, Lee J, Lam ET, Liachko I, Sullivan ST, Burton JN, Huson HJ, Nystrom JC, Kelley CM, Hutchison JL, Zhou Y, Sun J, Crisa A, Ponce De Leon FA, Schwartz JC, Hammond JA, Waldbieser GC, Schroeder SG, Liu GE, Dunham MJ, Shendure J, Sonstegard TS, Phillippy AM, Van Tassell CP, Smith TP. 2017. Single-molecule sequencing and chromatin conformation capture enable de novo reference assembly of the domestic goat genome. *Nature Genetics*, **49**(4): 643–650.

Blix AS. 2016. Adaptations to polar life in mammals and birds. *Journal of Experimental Biology*, **219**(8): 1093–1105.

Boocock GRB, Morrison JA, Popovic M, Richards N, Ellis L, Durie PR, Rommens JM. 2003. Mutations in SBDS are associated with shwachman-diamond syndrome. *Nature Genetics*, **33**(1): 97–101.

Bovine Genome Sequencing and Analysis Consortium, Elsik CG, Tellam RL, Worley KC, Gibbs RA, Muzny DM, Weinstock GM, Adelson DL, Eichler EE, Elnitski L, Guigo R, Hamernik DL, Kappes SM, Lewin HA, Lynn DJ, Nicholas FW, Reymond A, Rijnkels M, Skow LC, Zdobnov EM, Schook L, Womack J, Alioto T, Antonarakis SE, Astashyn A, Chapple CE, Chen HC, Chrast J, Câmara F, Ermolaeva O, Henrichsen CN, Hlavina W, Kapustin Y, Kiryutin B, Kitts P, Kokocinski F, Landrum M, Maglott D, Pruitt K, Sapojnikov V, Searle SM, Solovyev V, Souvorov A, Ucla C, Wyss C, Anzola JM, Gerlach D, Elhaik E, Graur D, Reese JT, Edgar RC, Mcewan JC, Payne GM, Raison JM, Junier T, Kriventseva EV, Eyras E, Plass M, Donthu R, Larkin DM, Reecy J, Yang MQ, Chen L, Cheng Z, Chitko-Mckown CG, Liu GE, Matukumalli LK, Song J, Zhu B, Bradley DG, Brinkman FS, Lau LP, Whiteside MD, Walker A, Wheeler TT, Casey T, German JB, Lemay DG, Maqbool NJ, Molenaar AJ, Seo S, Stothard P, Baldwin CL, Baxter R, Brinkmeyer-Langford CL, Brown WC, Childers CP, Connelley T, Ellis SA, Fritz K, Glass EJ, Herzig CT, Iivanainen A, Lahmers KK, Bennett AK, Dickens CM, Gilbert JG, Hagen DE, Salih H, Aerts J, Caetano AR, Dalrymple B, Garcia JF, Gill CA, Hiendleder SG, Memili E, Spurlock D, Williams JL, Alexander L, Brownstein MJ, Guan L, Holt RA, Jones SJ, Marra MA, Moore R, Moore SS, Roberts A, Taniguchi M, Waterman RC, Chacko J, Chandrabose MM, Cree A, Dao MD, Dinh HH, Gabisi RA, Hines S, Hume J, Jhangiani SN, Joshi V, Kovar CL, Lewis LR, Liu YS, Lopez J, Morgan MB, Nguyen NB, Okwuonu GO, Ruiz SJ, Santibanez J, Wright RA, Buhay C, Ding Y, Dugan-Rocha S, Herdandez J, Holder M, Sabo A, Egan A, Goodell J, Wilczek-Boney K, Fowler GR, Hitchens ME, Lozado RJ, Moen C, Steffen D, Warren JT, Zhang J, Chiu R, Schein JE, Durbin KJ, Havlak P, Jiang H, Liu Y, Qin X, Ren Y, Shen Y, Song H, Bell SN, Davis C, Johnson AJ, Lee S, Nazareth LV, Patel BM, Pu LL, Vattathil S, Williams RL Jr., Curry S, Hamilton C, Sodergren E, Wheeler DA, Barris W, Bennett GL, Eggen A, Green RD, Harhay GP, Hobbs M, Jann O, Keele JW, Kent MP, Lien S, Mckay SD, Mcwilliam S, Ratnakumar A, Schnabel RD, Smith T, Snelling WM, Sonstegard TS, Stone RT, Sugimoto Y, Takasuga A, Taylor JF, Van Tassell CP, Macneil MD, Abatepaulo AR, Abbey CA, Ahola V, Almeida IG, Amadio AF, Anatriello E, Bahadue SM, Biase FH, Boldt CR, Carroll JA, Carvalho WA, Cervelatti EP, Chacko E, Chapin JE, Cheng Y, Choi J, Colley AJ, De Campos TA, De Donato M, Santos IK, De Oliveira CJ, Deobald H, Devinoy E, Donohue KE, Dovc P, Eberlein A, Fitzsimmons CJ, Franzin AM, Garcia GR, Genini S, Gladney CJ, Grant JR, Greaser ML, Green JA, Hadsell DL, Hakimov HA, Halgren R, Harrow JL, Hart EA, Hastings N, Hernandez M, Hu ZL, Ingham A, Iso-Touru T, Jamis C, Jensen K, Kapetis D, Kerr T, Khalil SS, Khatib H, Kolbehdari D, Kumar CG, Kumar D, Leach R, Lee JC, Li C, Logan KM, Malinverni R, Marques E, Martin WF, Martins NF, Maruyama SR, Mazza R, Mclean KL, Medrano JF, Moreno BT, MoréDD, Muntean CT, Nandakumar HP, Nogueira MF, Olsaker I, Pant SD, Panzitta F, Pastor RC, Poli MA, Poslusny N, Rachagani S, Ranganathan S, Razpet A, Riggs PK, Rincon G, Rodriguez-Osorio N, Rodriguez-Zas SL, Romero NE, Rosenwald A, Sando L, Schmutz SM, Shen L, Sherman L, Southey BR, Lutzow YS, Sweedler JV, Tammen I, Telugu BP, Urbanski JM, Utsunomiya YT, Verschoor CP, Waardenberg AJ, Wang Z, Ward R, Weikard R, Welsh TH Jr., White SN, Wilming LG, Wunderlich KR, Yang J, Zhao FQ. 2009. The genome sequence of taurine cattle: a window to ruminant biology and evolution. *Science*, **324**(5926): 522–528.

Bubenik GA, Bubenik AB. 2012. Horns, Pronghorns, and Antlers: Evolution, Morphology, Physiology, and Social Significance. Springer Science & Business Media.

Canavez FC, Luche DD, Stothard P, Leite KRM, Sousa-Canavez JM, Plastow G, Meidanis J, Souza MA, Feijao P, Moore SS, Camara-Lopes LH. 2012. Genome sequence and assembly of *Bos indicus*. *Journal of Heredity*, **103**(3): 342–348.

Castelló JR. 2016. Bovids of the World: Antelopes, Gazelles, Cattle, Goats, Sheep, and Relatives. Princeton University Press.

Chen L, Qiu Q, Jiang Y, Wang K, Lin Z, Li Z, Bibi F, Yang Y, Wang J, Nie W, Su W, Liu G, Li Q, Fu W, Pan X, Liu C, Yang J, Zhang C, Yin Y, Wang Y, Zhao Y, Zhang C, Wang Z, Qin Y, Liu W, Wang B, Ren Y, Zhang R, Zeng Y, Da Fonseca RR, Wei B, Li R, Wan W, Zhao R, Zhu W, Wang Y, Duan S, Gao Y, Zhang YE, Chen C, Hvilsom C, Epps CW, Chemnick LG, Dong Y, Mirarab S, Siegismund HR, Ryder OA, Gilbert MTP, Lewin HA, Zhang G, Heller R, Wang W. 2019. Large-scale ruminant genome sequencing provides insights into their evolution and distinct traits. *Science*, 364(6446).

Chen N, Cai Y, Chen Q, Li R, Wang K, Huang Y, Hu S, Huang S, Zhang H, Zheng Z, Song W, Ma Z, Ma Y, Dang R, Zhang Z, Xu L, Jia Y, Liu S, Yue X, Deng W, Zhang X, Sun Z, Lan X, Han J, Chen H, Bradley DG, Jiang Y, Lei C. 2018. Whole-genome resequencing reveals world-wide ancestry and adaptive introgression events of domesticated cattle in east asia. *Nature Communications*, **9**(1): 2337.

Clauss M, Frey R, Kiefer B, Lechner-Doll M, Loehlein W, Polster C, Rossner GE, Streich WJ. 2003. The maximum attainable body size of herbivorous mammals: morphophysiological constraints on foregut, and adaptations of hindgut fermenters. *Oecologia*, **136**(1): 14–27.

Clauss M, Rössner GE. 2014. Old world ruminant morphophysiology, life history, and fossil record: exploring key innovations of a diversification sequence. *Annales Zoologici Fennici*, **51**(1–2): 80–94.

Daetwyler HD, Capitan A, Pausch H, Stothard P, Van Binsbergen R, Brondum RF, Liao X, Djari A, Rodriguez SC, Grohs C, Esquerré D, Bouchez O, Rossignol MN, Klopp C, Rocha D, Fritz S, Eggen A, Bowman PJ, Coote D, Chamberlain AJ, Anderson C, Vantassell CP, Hulsegge I, Goddard ME, Guldbrandtsen B, Lund MS, Veerkamp RF, Boichard DA, Fries R, Hayes BJ. 2014. Whole-genome sequencing of 234 bulls facilitates mapping of monogenic and complex traits in cattle. *Nature Genetics*, **46**(8): 858–865.

Davis EB, Brakora KA, Lee AH. 2011. Evolution of ruminant headgear: a review. *Proceedings of the Royal Society B: Biological Sciences*, **278**(1720): 2857–2865.

Decker JE, Pires JC, Conant GC, Mckay SD, Heaton MP, Chen K, Cooper A, Vilkki J, Seabury CM, Caetano AR, Johnson GS, Brenneman RA, Hanotte O, Eggert LS, Wiener P, Kim JJ, Kim KS, Sonstegard TS, Van Tassell CP, Neibergs HL, Mcewan JC, Brauning R, Coutinho LL, Babar ME, Wilson GA, Mcclure MC, Rolf MM, Kim J, Schnabel RD, Taylor JF. 2009. Resolving the evolution of extant and extinct ruminants with high-throughput phylogenomics. *Proceedings of the National Academy of Sciences of the Untied States of America*, **106**(44): 18644–18649.

Dong Y, Zhang X, Xie M, Arefnezhad B, Wang Z, Wang W, Feng S, Huang G, Guan R, Shen W, Bunch R, Mcculloch R, Li Q, Li B, Zhang G, Xu X, Kijas JW, Salekdeh GH, Wang W, Jiang Y. 2015. Reference genome of wild goat (*Capra aegagrus*) and sequencing of goat breeds provide insight into genic basis of goat domestication. *BMC Genomics*, **16**: 431.

Dos Reis M, Inoue J, Hasegawa M, Asher RJ, Donoghue PCJ, Yang Z. 2012. Phylogenomic datasets provide both precision and accuracy in estimating the timescale of placental mammal phylogeny. *Proceedings of the Royal Society B: Biological Sciences*, **279**(1742): 3491–3500.

Gatesy J, Yelon D, Desalle R, Vrba ES. 1992. Phylogeny of the bovidae (Artiodactyla, Mammalia), based on mitochondrial ribosomal DNA sequences. *Molecular Biology and Evolution*, **9**(3): 433–446.

Ge RL, Cai Q, Shen YY, San A, Ma L, Zhang Y, Yi X, Chen Y, Yang L, Huang Y, He R, Hui Y, Hao M, Li Y, Wang B, Ou X, Xu J, Zhang Y, Wu K, Geng C, Zhou W, Zhou T, Irwin DM, Yang Y, Ying L, Bao H, Kim J, Larkin DM, Ma J, Lewin HA, Xing J, Platt RN 2nd , Ray DA, Auvil L, Capitanu B, Zhang X, Zhang G, Murphy RW, Wang J, Zhang YP, Wang J. 2013. Draft genome sequence of the Tibetan antelope. *Nature Communications*, **4**: 1858.

Goss RJ. 1983. Deer Antlers: Regeneration, Function and Evolution. Academic Press.

Griner LA. 1983. Pathology of Zoo Animals. A Review of Necropsies Conducted Over a Fourteen-Year Period at the San Diego Zoo and San Diego Wild Animal Park. San Diego, California: Zoological Society, PO Box 551.

Grobet L, Martin LJ, Poncelet D, Pirottin D, Brouwers B, Riquet J, Schoeberlein A, Dunner S, Ménissier F, Massabanda J, Fries R, Hanset R, Georges M. 1997. A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. *Nature Genetics*, **17**(1): 71–74.

Gu H, Cao Y, Qiu B, Zhou Z, Deng R, Chen Z, Li R, Li X, Wei Q, Xia X, Yong W. 2016. Establishment and phenotypic analysis of an *Mstn* knockout rat. *Biochemical and Biophysical Research Communications*, **477**(1): 115–122.

Hassanin A, Delsuc F, Ropiquet A, Hammer C, Jansen Van Vuuren B, Matthee C, Ruiz-Garcia M, Catzeflis F, Areskoug V, Nguyen TT, Couloux A. 2012. Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. *Comptes Rendus Biologies*, **335**(1): 32–50.

Hassanin A, Douzery EJP. 2003. Molecular and morphological phylogenies of Ruminantia and the alternative position of the Moschidae. *Systematic Biology*, **52**(2): 206–228.

Heller R, Frandsen P, Lorenzen ED, Siegismund HR. 2013. Are there really twice as many bovid species as we thought?. *Systematic Biology*, **62**(3): 490–493.

Hernández Fernández M, Vrba ES. 2005. A complete estimate of the phylogenetic relationships in Ruminantia: a dated species-level supertree of the extant ruminants. *Biological Reviews Cambrideg Philosophical Society*, **80**(2): 269–302.

Hu XJ, Yang J, Xie XL, Lv FH, Cao YH, Li WR, Liu MJ, Wang YT, Li JQ, Liu YG, Ren YL, Shen ZQ, Wang F, Hehua E, Han JL, Li MH, Nielsen R. 2019. The genome landscape of Tibetan sheep reveals adaptive introgression from argali and the history of early human settlements on the Qinghai–Tibetan Plateau. *Molecular Biology and Evolution*, **36**(2): 283–303.

Irwin DM. 2015. Genomic organization and evolution of ruminant lysozyme c genes. *Zoological Research*, **36**(1): 1–17.

Janis C. 1976. The evolutionary strategy of the Equidae and the origins of rumen and cecal digestion. *Evolution*, **30**(4): 757–774.

Janis CM. 1987. Grades and clades in hornless ruminant evolution: the reality of the Gelocidae and the systematic position of *Lophiomeryx* and *Bachitherium*. *Journal of Vertebrate Paleontology*, **7**(2): 200–216.

Janis CM, Theodor JM. 2014. Cranial and postcranial morphological data in ruminant phylogenetics. *Zitteliana*, **32**: 15–31.

Jarvis ED, Mirarab S, Aberer AJ, Li B, Houde P, Li C, Ho SY, Faircloth BC, Nabholz B, Howard JT, Suh A, Weber CC, Da Fonseca RR, Li J, Zhang F, Li H, Zhou L, Narula N, Liu L, Ganapathy G, Boussau B, Bayzid MS, Zavidovych V, Subramanian S, Gabaldon T, Capella-Gutiérrez S, Huerta-Cepas J, Rekepalli B, Munch K, Schierup M, Lindow B, Warren WC, Ray D, Green RE, Bruford MW, Zhan X, Dixon A, Li S, Li N, Huang Y, Derryberry EP, Bertelsen MF, Sheldon FH, Brumfield RT, Mello CV, Lovell PV, Wirthlin M, Schneider MP, Prosdocimi F, Samaniego JA, Vargas Velazquez AM, Alfaro-Núñez A, Campos PF, Petersen B, Sicheritz-Ponten T, Pas A, Bailey T, Scofield P, Bunce M, Lambert DM, Zhou Q, Perelman P, Driskell AC, Shapiro B, Xiong Z, Zeng Y, Liu S, Li Z, Liu B, Wu K, Xiao J, Yinqi X, Zheng Q, Zhang Y, Yang H, Wang J, Smeds L, Rheindt FE, Braun M, Fjeldsa J, Orlando L, Barker FK, Jønsson KA, Johnson W, Koepfli KP, O'brien S, Haussler D, Ryder OA, Rahbek C, Willerslev E, Graves GR, Glenn TC, Mccormack J, Burt D, Ellegren H, Alström P, Edwards SV, Stamatakis A,

Mindell DP, Cracraft J, Braun EL, Warnow T, Wang J, Gilbert MT, Zhang G. 2014. Whole-genome analyses resolve early branches in the tree of life of modern birds. *Science*, **346**(6215): 1320–1331.

Jiang Y, Xie M, Chen W, Talbot R, Maddox JF, Faraut T, Wu C, Muzny DM, Li Y, Zhang W, Stanton JA, Brauning R, Barris WC, Hourlier T, Aken BL, Searle SMJ, Adelson DL, Bian C, Cam GR, Chen Y, Cheng S, Desilva U, Dixen K, Dong Y, Fan G, Franklin IR, Fu S, Guan R, Highland MA, Holder ME, Huang G, Ingham AB, Jhangiani SN, Kalra D, Kovar CL, Lee SL, Liu W, Liu X, Lu C, Lv T, Mathew T, Mcwilliam S, Menzies M, Pan S, Robelin D, Servin B, Townley D, Wang W, Wei B, White SN, Yang X, Ye C, Yue Y, Zeng P, Zhou Q, Hansen JB, Kristensen K, Gibbs RA, Flicek P, Warkup CC, Jones HE, Oddy VH, Nicholas FW, Mcewan JC, Kijas J, Wang J, Worley KC, Archibald AL, Cockett N, Xu X, Wang W, Dalrymple BP. 2014. The sheep genome illuminates biology of the rumen and lipid metabolism. *Science*, **344**(6188): 1168–1173.

Koepfli KP, Tamazian G, Wildt D, Dobrynin P, Kim C, Frandsen PB, Godinho R, Yurchenko AA, Komissarov A, Krasheninnikova K, Kliver S, Kolchanova S, Goncalves M, Carneiro M, Pinto PV, Ferrand N, Maldonado JE, Ferrie GM, Chemnick L, Ryder OA, Johnson WE, Comizzoli P, O'brien SJ, Pukazhenthi BS. 2019. Whole genome sequencing and re-sequencing of the sable antelope (*Hippotragus niger*): a resource for monitoring diversity in *ex situ* and *in situ* populations. *G3*: Genes, Genomes, Genetics, **9**(6): 1785–1793.

Kropatsch R, Dekomien G, Akkad DA, Gerding WM, Petrasch-Parwez E, Young ND, Altmuller J, Nürnberg P, Gasser RB, Epplen JT. 2013. SOX9 duplication linked to intersex in deer. *PLoS One*, **8**(9): e73734.

Li H, Wang G, Hao Z, Zhang G, Qing Y, Liu S, Qing L, Pan W, Chen L, Liu G, Zhao R, Jia B, Zeng L, Guo J, Zhao L, Zhao H, Lv C, Xu K, Cheng W, Li H, Zhao HY, Wang W, Wei HJ. 2016. Generation of biallelic knock-out sheep via gene-editing and somatic cell nuclear transfer. *Scientific Reports*, **6**: 33675.

Li Z, Lin Z, Ba H, Chen L, Yang Y, Wang K, Qiu Q, Wang W, Li G. 2017. Draft genome of the reindeer (*Rangifer tarandus*). *Gigascience*, **6**(12): 1–5

Lin Z, Chen L, Chen X, Zhong Y, Yang Y, Xia W, Liu C, Zhu W, Wang H, Yan B, Yang Y, Liu X, Sternang Kvie K, Roed KH, Wang K, Xiao W, Wei H, Li G, Heller R, Gilbert MTP, Qiu Q, Wang W, Li Z. 2019. Biological adaptations in the Arctic cervid, the reindeer (*Rangifer tarandus*). *Science*, **364**(6446): eaav6312.

Lombard LS, Witte EJ. 1959. Frequency and types of tumors in mammals and birds of the philadelphia zoological garden. *Cancer Research*, **19**(2): 127–141.

Lu W, Meng QJ, Tyler NJC, Stokkan KA, Loudon ASI. 2010. A circadian clock is not required in an arctic mammal. *Current Biology*, **20**(6): 533–537.

Luo J, Song Z, Yu S, Cui D, Wang B, Ding F, Li S, Dai Y, Li N. 2014. Efficient generation of myostatin (MSTN) biallelic mutations in cattle using zinc finger nucleases. *PLoS One*, **9**(4): e95225.

Medugorac I, Graf A, Grohs C, Rothammer S, Zagdsuren Y, Gladyr E, Zinovieva N, Barbieri J, Seichter D, Russ I, Eggen A, Hellenthal G, Brem G, Blum H, Krebs S, Capitan A. 2017. Whole-genome analysis of introgressive hybridization and characterization of the bovine legacy of mongolian yaks. *Nature Genetics*, **49**(3): 470–475.

Millen DD, Arrigoni MDB, Pacheco RDL. 2016. Rumenology. Cham: Springer International Publishing.

Mintoo AA, Zhang H, Chen C, Moniruzzaman M, Deng T, Anam M, Emdadul

Huque QM, Guang X, Wang P, Zhong Z, Han P, Khatun A, Awal TM, Gao Q, Liang X. 2019. Draft genome of the river water buffalo. *Ecology and Evolution*, **9**(6): 3378–3388.

Naval-Sanchez M, Nguyen Q, Mcwilliam S, Porto-Neto LR, Tellam R, Vuocolo T, Reverter A, Perez-Enciso M, Brauning R, Clarke S, Mcculloch A, Zamani W, Naderi S, Rezaei HR, Pompanon F, Taberlet P, Worley KC, Gibbs RA, Muzny DM, Jhangiani SN, Cockett N, Daetwyler H, Kijas J. 2018. Sheep genome functional annotation reveals proximal regulatory elements contributed to the evolution of modern breeds. *Nature Communications*, **9**(1): 859.

Qanbari S, Pausch H, Jansen S, Somel M, Strom TM, Fries R, Nielsen R, Simianer H. 2014. Classic selective sweeps revealed by massive sequencing in cattle. *PLoS Genetics*, **10**(2): e1004148.

Qiu Q, Wang L, Wang K, Yang Y, Ma T, Wang Z, Zhang X, Ni Z, Hou F, Long R, Abbott R, Lenstra J, Liu J. 2015. Yak whole-genome resequencing reveals domestication signatures and prehistoric population expansions. *Nature Communications*, **6**: 10283.

Qiu Q, Zhang GJ, Ma T, Qian WB, Wang JY, Ye ZQ, Cao CC, Hu QJ, Kim J, Larkin DM, Auvil L, Capitanu B, Ma J, Lewin HA, Qian XJ, Lang YS, Zhou R, Wang LZ, Wang K, Xia JQ, Liao SG, Pan SK, Lu X, Hou HL, Wang Y, Zang XT, Yin Y, Ma H, Zhang J, Wang ZF, Zhang YM, Zhang DW, Yonezawa T, Hasegawa M, Zhong Y, Liu WB, Zhang Y, Huang ZY, Zhang SX, Long RJ, Yang HM, Wang J, Lenstra JA, Cooper DN, Wu Y, Wang J, Shi P, Wang J, Liu JQ. 2012. The yak genome and adaptation to life at high altitude. *Nature Genetics*, **44**(8): 946–949.

Russell JB, Rychlik JL. 2001. Factors that alter rumen microbial ecology. *Science*, **292**(5519): 1119–1122.

Russell T, Cullingham C, Kommadath A, Stothard P, Herbst A, Coltman D. 2019. Development of a novel mule deer genomic assembly and species-diagnostic SNP panel for assessing introgression in mule deer, white-tailed deer, and their interspecific hybrids. *G3: Genes, Genomes, Genetics,* **9**(3): 911–919.

Sánchez IM, Domingo MS, Morales J. 2010. The genus *Hispanomeryx* (Mammalia, Ruminantia, Moschidae) and its bearing on musk deer phylogeny and systematics. *Palaeontology*, **53**(5): 1023–1047.

Sartori R, Schirwis E, Blaauw B, Bortolanza S, Zhao J, Enzo E, Stantzou A, Mouisel E, Toniolo L, Ferry A, Stricker S, Goldberg AL, Dupont S, Piccolo S, Amthor H, Sandri M. 2013. BMP signaling controls muscle mass. *Nature Genetics*, **45**(11): 1309–1318.

Seabury CM, Bhattarai EK, Taylor JF, Viswanathan GG, Cooper SM, Davis DS, Dowd SE, Lockwood ML, Seabury PM. 2011. Genome-wide polymorphism and comparative analyses in the white-tailed deer (*Odocoileus virginianus*): a model for conservation genomics. *PLoS One*, **6**(1): e15811.

Seshadri R, Leahy SC, Attwood GT, Teh KH, Lambie SC, Cookson AL, Eloe-Fadrosh EA, Pavlopoulos GA, Hadjithomas M, Varghese NJ, Paez-Espino D, Hungate Project C, Perry R, Henderson G, Creevey CJ, Terrapon N, Lapebie P, Drula E, Lombard V, Rubin E, Kyrpides NC, Henrissat B, Woyke T, Ivanova NN, Kelly WJ. 2018. Cultivation and sequencing of rumen microbiome members from the hungate1000 collection. *Nature Biotechnology*, **36**(4): 359–367.

Shenolikar S, Voltz JW, Minkoff CM, Wade JB, Weinman EJ. 2002. Targeted disruption of the mouse NHERF-1 gene promotes internalization of proximal tubule sodium-phosphate cotransporter type IIa and renal

phosphate wasting. *Proceedings of the National Academy of Sciences of the Untied States of America*, **99**(17): 11470–11475.

Shi L. 1983. Sex-linked chromosome polymorphism in black muntjac, Muntiacus crinifrons. In: Swaminathan MS. Proceedings of the Fifth International Congress of Genetics. New Dehli, 153.

Simonson TS, Yang Y, Huff CD, Yun H, Qin G, Witherspoon DJ, Bai Z, Lorenzo FR, Xing J, Jorde LB, Prchal JT, Ge R. 2010. Genetic evidence for high-altitude adaptation in Tibet. *Science*, **329**(5987): 72–75.

Soubrier J, Gower G, Chen K, Richards SM, Llamas B, Mitchell KJ, Ho SY, Kosintsev P, Lee MSY, Baryshnikov G, Bollongino R, Bover P, Burger J, Chivall D, Crégut-Bonnoure E, Decker JE, Doronichev VB, Douka K, Fordham DA, Fontana F, Fritz C, Glimmerveen J, Golovanova LV, Groves C, Guerreschi A, Haak W, Higham T, Hofman-Kaminska E, Immel A, Julien MA, Krause J, Krotova O, Langbein F, Larson G, Rohrlach A, Scheu A, Schnabel RD, Taylor JF, Tokarska M, Tosello G, Van Der Plicht J, Van Loenen A, Vigne JD, Wooley O, Orlando L, Kowalczyk R, Shapiro B, Cooper A. 2016. Early cave art and ancient DNA record the origin of European bison. *Nature Communications*, **7**: 13158.

Stewart RD, Auffret MD, Warr A, Wiser AH, Press MO, Langford KW, Liachko I, Snelling TJ, Dewhurst RJ, Walker AW, Roehe R, Watson M. 2018. Assembly of 913 microbial genomes from metagenomic sequencing of the cow rumen. *Nature Communications*, **9**(1): 870.

Stokkan KA, Van Oort BE, Tyler NJC, Loudon ASI. 2007. Adaptations for life in the Arctic: evidence that melatonin rhythms in reindeer are not driven by a circadian oscillator but remain acutely sensitive to environmental photoperiod. *Journal of Pineal Research*, **43**(3): 289–293.

Van Oort BEH, Tyler NJC, Gerkema MP, Folkow L, Blix AS, Stokkan KA. 2005. Circadian organization in reindeer. *Nature*, **438**(7071): 1095–1096.

Wang K, Lenstra JA, Liu L, Hu Q, Ma T, Qiu Q, Liu J. 2018a. Incomplete lineage sorting rather than hybridization explains the inconsistent phylogeny of the wisent. *Communications Biology*, **1**: 169.

Wang K, Wang L, Lenstra JA, Jian J, Yang Y, Hu Q, Lai D, Qiu Q, Ma T, Du Z, Abbott R, Liu J. 2017a. The genome sequence of the wisent (*Bison bonasus*). *Gigascience*, **6**(4): 1–5.

Wang MS, Zeng Y, Wang X, Nie WH, Wang JH, Su WT, Otecko NO, Xiong ZJ, Wang S, Qu KX, Yan SQ, Yang MM, Wang W, Dong Y, Wu DD, Zhang YP. 2017b. Draft genome of the gayal, *Bos frontalis*. *Gigascience*, **6**(11): 1–7.

Wang X, Liu J, Zhou G, Guo J, Yan H, Niu Y, Li Y, Yuan C, Geng R, Lan X, An X, Tian X, Zhou H, Song J, Jiang Y, Chen Y. 2016. Whole-genome sequencing of eight goat populations for the detection of selection signatures underlying production and adaptive traits. *Scientific Reports*, 6(1.

Wang X, Niu Y, Zhou J, Zhu H, Ma B, Yu H, Yan H, Hua J, Huang X, Qu L, Chen Y. 2018b. CRISPR/Cas9-mediated *MSTN* disruption and heritable mutagenesis in goats causes increased body mass. *Animal Genetics*, **49**(1): 43–51.

Wang Y, Gao S, Zhao Y, Chen WH, Shao JJ, Wang NN, Li M, Zhou GX, Wang L, Shen WJ, Xu JT, Deng WD, Wang W, Chen YL, Jiang Y. 2019a. Allele-specific expression and alternative splicing in horse×donkey and cattle×yak hybrids. *Zoological Research*, **40**(4): 293–305.

Wang Y, Zhang C, Wang N, Li Z, Heller R, Liu R, Zhao Y, Han J, Pan X, Zheng Z, Dai X, Chen C, Dou M, Peng S, Chen X, Liu J, Li M, Wang K, Liu C, Lin Z, Chen L, Hao F, Zhu W, Song C, Zhao C, Zheng C, Wang J, Hu S, Li C, Yang H, Jiang L, Li G, Liu M, Sonstegard TS, Zhang G, Jiang Y, Wang W, Qiu Q. 2019b. Genetic basis of ruminant headgear and rapid antler regeneration. *Science*, 364(6446.

Warner ED. 1958. The organogenesis and early histogenesis of the bovine stomach. *American Journal of Anatomy*, **102**(1): 33–63.

Webb SD, Taylor BE. 1980. The phylogeny of hornless ruminants and a description of the cranium of *Archaeomeryx*. *Bulletin of the American Museum of Natural History*, **167**:117–158.

Wilkins AS, Wrangham RW, Fitch WT. 2014. The "domestication syndrome" in mammals: a unified explanation based on neural crest cell behavior and genetics. *Genetics*, **197**(3): 795–808.

Wu DD, Ding XD, Wang S, Wójcik JM, Zhang Y, Tokarska M, Li Y, Wang MS, Faruque O, Nielsen R, Zhang Q, Zhang YP. 2018. Pervasive introgression facilitated domestication and adaptation in the *Bos* species complex. *Nature Ecology & Evolution*, **2**(7): 1139–1145.

Wurster DH, Benirschke K. 1967. Chromosome studies in some deer, the springbok, and the pronghorn, with notes on placentation in deer. *Cytologia (Tokyo)*, **32**(2): 273–285.

Wurster DH, Benirschke K. 1970. Indian muntjac, *Muntiacus muntjak*: a deer with a low diploid chromosome number. *Science*, **168**(3937): 1364–1366.

Xiang R, Oddy VH, Archibald AL, Vercoe PE, Dalrymple BP. 2016. Epithelial, metabolic and innate immunity transcriptomic signatures differentiating the rumen from other sheep and mammalian gastrointestinal tract tissues. *Peer J*, **4**: e1762.

Yang Y, Wang Y, Zhao Y, Zhang X, Li R, Chen L, Zhang G, Jiang Y, Qiu Q, Wang W, Wei HJ, Wang K. 2017. Draft genome of the marco polo sheep (*Ovis ammon polii*). *Gigascience*, **6**(12): 1–7.

Zhang C, Chen L, Zhou Y, Wang K, Chemnick LG, Ryder OA, Wang W, Zhang G, Qiu Q. 2018. Draft genome of the milu (*Elaphurus davidianus*). *Gigascience*, **7**(2). doi: 10.1093/gigascience/gix130.

Zhang Z, Xu D, Wang L, Hao J, Wang J, Zhou X, Wang W, Qiu Q, Huang X, Zhou J, Long R, Zhao F, Shi P. 2016. Convergent evolution of rumen microbiomes in high-altitude mammals. *Current Biology*, **26**(14): 1873–1879.